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Microparticulated whey protein addition modulates rheological and microstructural properties of high-protein acid milk gels

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ABSTRACT

The demand for low-fat, high-protein dairy products, such as yoghurts and fresh cheeses is growing considerably. However, reduction of fat, with a concomitant increase in protein, contributes to textural and functional challenges in the finished products. The aim of this study was to investigate how the rheological, microstructural, textural and water holding properties of acid-induced milk protein gels are influenced by the addition of a commercial protein-based fat replacer (Simplesse® 100), i.e., microparticulated whey protein (MWP). MWP was added to fat-free, high-protein samples (<0.3%, w/w, fat; 8%, w/w, protein) at different concentrations (0–4.4%, w/w). The gel properties were significantly influenced by the addition of MWP, due to the prevention of casein aggregation and creation of serum channels. The gels containing MWP had lower storage modulus, lower yield stress, lower firmness, higher porosity, lower tortuosity and lower water holding capacity compared with the control gel (without MWP).

1. Introduction

The demand for low-fat, high-protein variants of dairy products, such as yoghurts and cheeses, has increased dramatically over the last 20 years (Drake & Swanson, 1995; Purwanti, van der Goot, Boom, & Vereijken, 2010; Rodríguez, 1998). However, such products have textural, rheological and microstructural properties that are very different from those of their full-fat counterparts. For instance, the removal, or reduction, of fat causes several challenges with acceptability of the final products, such as poor texture, lack of flavour and undesirable colour (Mistry, 2001). In addition, the increase in protein content leads to strong and tight networks due to a reduction of the distance between the protein particles in the matrix, resulting in products that are firmer and have a more rubbery mouth-feel than their full-fat equivalents (Chever, Guyomarc'h, Beaucher, & Famelart, 2014; Purwanti et al., 2010).

Three main approaches have been investigated to address the challenges associated with fat removal or reduction in dairy products (Drake & Swanson, 1995; Mistry, 2001; Tufeanu & Tiţa, 2016): (a) modification of conventional dairy processing technologies to improve moisture retention and/or increase the surface area of fat globules during homogenisation; (b) use of adjunct cultures to improve flavour development; (c) use of fat replacers to improve texture and flavour. These approaches result in modification of the intermediate acid- or rennet-induced gel microstructure and, ultimately, in the physical and organoleptic properties of the final product.

Fat replacers may be divided into two categories: (a) fat substitutes, which are fat-based materials and (b) fat mimetics, which are generally polar, water-soluble compounds and may be protein- or carbohydrate-based. Protein-based ingredients, such as microparticulated whey protein (MWP), are receiving increasing attention due to the interest

in adding value to whey produced by the dairy industry and to the nutritional contribution of the added whey proteins (Hinrichs, 2001; Smithers, 2015). Different authors have suggested the use of MWP as a fat replacer to provide desirable physical and organoleptic properties in low-fat dairy products such as ice creams (Karaca, Guven, Yasar, Kaya, & Kahyaoglu, 2009; Yilsay, Yilmaz, & Bayizit, 2006), stirred and set-style yoghurts (Barrantes, Tamime, Muir, & Sword, 1994; Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004; Tamime, Kaláb, Muir, & Barrantes, 1995; Torres, Janhøj, Mikkelsen, & Ipsen, 2011; Torres, Mutaf, Larsen, & Ipsen, 2016) and cheeses (Lee, Huss, Klostermeyer, & Anema, 2013; McMahon, Alleyne, Fife, & Oberg, 1996; Romeih, Michaelidou, Biliaderis, & Zerfiridis, 2002; Sahan, Yasar, Hayaloglu, Karaca, & Kaya, 2008; Schenkel, Samudrala, & Hinrichs, 2013; Sturaro, De Marchi, Zorzi, & Cassandro, 2015), by increasing moisture retention, thereby improving texture and yield. More recently, a study showed that micro-structuring of whey proteins, and the use of these protein particles that mimic fat-like properties, is an interesting approach to modulate the textural properties of high-protein foods (Purwanti, Peters, & van der Goot, 2013).

However, in comparison with the applications above, the use of MWP as a fat replacer or as a protein micro-structuring tool in high-protein fresh dairy systems has received much less attention. Most studies in this area have been conducted in cheese products, after whey separation, brining (Kavas, Oysun, Kinik, & Uysal, 2004; Koca & Metin, 2004; Lobato-Calleros et al., 2007) and ripening (McMahon et al., 1996). Other studies have been conducted in low-protein yoghurt products (Sandoval-Castilla et al., 2004; Tamime et al., 1995). Therefore, the effect of MWP on the acid gelation, and on the physical properties of the resulting (in particular, high-protein) acid milk gels is extremely limited. In this context, the objective of this study was to investigate how the addition of a commercial MWP ingredient (Simplesse® 100) at different concentrations (from 0 to 4.4%, w/w) to fat-

free, high-protein formulations (<0.3% fat; 8% protein) influenced the acid gelation of the protein suspensions and the rheological, microstructural, textural and water holding properties of the resulting acid milk gels. This commercial ingredient is a natural dairy ingredient made from whey protein concentrate that undergoes a microparticulation process by simultaneous heating and shearing.

2. Materials and methods

2.1. Milk protein ingredients

The ingredients used in this study were as follows: medium-heat skim milk powder (SMP; Westbury Dairies Ltd., Wiltshire, UK), milk protein concentrate (MPC; Délicelait Normandie, Moyon, France) and Simplesse® 100 microparticulated whey protein concentrate (MWP; CP Kelco, Atlanta, GA, USA). The concentrations of protein, lactose, fat, moisture and ash were, respectively: 33.0, 56.6, 0.6, 3.5 and 6.3% (w/w) for SMP; 70.0, 17.0, 1.2, 4.0 and 7.8 (w/w) for MPC and 52.5, 35.1, 4.1, 3.6 and 5.0% (w/w) for MWP.

2.2. Preparation of reconstituted milk protein suspensions

Five milk protein suspensions were prepared from different blends of the milk protein ingredients: control (C) (without MWP addition); low MWP (LS) (0.55%, w/w, MWP), medium MWP (MS) (1.10%, w/w, MWP), high MWP (HS) (2.20%, w/w, MWP) and total MWP (TS) (4.40%, w/w, MWP). The incorporation levels for the MWP were based on recommended use levels by the manufacturer (0.5 to ~4%, w/w). The level of addition of SMP was maintained constant while the quantities of both MWP and MPC were varied to

keep the protein content of the system constant at 8%, w/w (i.e., MWP replaced MPC). The protein composition of the milk protein suspensions is presented in Table 1. The ingredients were reconstituted in deionised water under constant magnetic stirring at 22 °C and sodium azide was added to the milk protein suspensions (0.02%, w/w) to prevent bacterial growth. The pH of the suspensions was adjusted to 6.6 using 1 M HCl and/or 1 M NaOH and they were equilibrated at 4 °C for 18 h to ensure complete rehydration.

2.3. Viscosity measurements in unheated reconstituted milk protein suspensions

Viscosity of the unheated reconstituted milk protein suspensions was measured using a Discovery Hybrid Rheometer (TA Instruments, Crawley, UK) equipped with a concentric cylinder geometry (internal diameter = 25 mm; external diameter = 27.5 mm). Samples (25 g) were loaded and allowed to equilibrate at 20 °C for 2 min, before being pre-sheared at 250 s^{-1} for 1 min and were then equilibrated at 0 s^{-1} for 1 min. After this, the shear stress and apparent viscosity were monitored over a shear rate range from 10 to 500 s^{-1} over 2 min at 20 °C. The average apparent viscosity at 500 s^{-1} was recorded.

2.4. Particle size measurements in unheated reconstituted milk protein suspensions

The size of particles in the reconstituted milk protein suspensions were measured by dynamic light scattering (DLS) using a Zetasizer (Malvern Instruments Ltd., Malvern, UK). Before analysis, the initial reconstituted suspensions were diluted 1:100 with ultrapure water. The parameters used were as follows: material refractive index, 1.45; dispersant refractive index, 1.33; material absorption, 0.001. The experiments were carried out at 25 °C and five

scans were completed per run in 3 independent samples for each reconstituted milk protein suspension.

2.5. Heat treatment of reconstituted milk protein suspensions

A high-temperature short-time (HTST) heating process was conducted at laboratory-scale using a TA Instruments ARG2 controlled-stress rheometer (TA Instruments, Crawley, UK) equipped with a starch pasting cell geometry and TRIOS v.8.32 software. The pH of suspensions was adjusted to 6.6, if necessary, before heat treatment. The suspensions were equilibrated at 20 °C for 2 min, and the temperature was then increased at 10 °C min^{-1} to 80 °C, with a holding time of 106 s at 80 °C before being reduced at 10 °C min^{-1} to 30 °C, followed by a hold time of 5 min at 30 °C. A shear rate of 15 s^{-1} was applied and viscosity was recorded during heating, holding and cooling. At least three replicates for each milk protein suspension were subjected to this heat treatment.

2.6. Production of acid milk gels from heated milk protein suspensions

Acid milk gels were produced by acidification of the heated milk protein suspensions with 2.5% (w/w) glucono-γ-lactone (GdL, Sigma-Aldrich, St. Louis, MO, USA). Samples were kept at 30 °C for up to 8 h until a pH of 4.8 was attained. The pH of the suspensions was monitored over time during acidification using a pH meter.

2.7. Rheological properties of acid milk gels

The rheological properties of acid gel formation were monitored by small amplitude oscillatory rheology (SAOR) using a Discovery Hybrid Rheometer (TA Instruments) equipped with a concentric cylinder geometry (internal diameter = 25 mm; external diameter = 27.5 mm). The test conditions used were frequency of 0.1 Hz, maximum strain of 1%, and were previously established to be within the linear viscoelastic region for these samples. Storage modulus (G′) values were recorded until pH 4.8 was attained. Gelation was defined as the time post GdL addition at which the G′ value of the gel exceeded 1 Pa. The large deformation properties of acid milk gels formed in situ were determined by applying a single, constant low shear rate (0.01 s^{-1}) up to the yielding of the gel (yield stress and yield strain determination). The apparent stress and strain at yield were defined as the point when shear stress started to decrease. Measurements were taken in triplicate for each milk protein system.

2.8. Microstructural characterisation of acid milk gels

The microstructural observations were performed using an inverted Olympus FluoView® FV1000 confocal laser scanning microscope (CLSM) (Olympus America Inc., Melville, NY, USA). Fast Green FCF (Sigma-Aldrich) was used to label the protein phase of the systems to observe the protein network of the resulting acid milk gels. Fast Green FCF dissolved in water (0.1%, w/v) was added to the heat-treated suspensions just after the addition of GdL (2.5%, w/w) to reach a final concentration of 0.005% (w/v). The mixture (1 mL) was transferred to CoverWell™ perfusion chambers (PCL1L-2.5; Grace Bio-Labs, Bend, OR, USA) and the perfusion chambers were incubated at 30 °C until the target pH of 4.8 was attained. This system enabled in situ and non-destructive examination of the microstructure of the acid milk gels. Representative images of each sample were taken using a 60× oil immersion objective (numerical aperture = 1.4) at emission wavelengths of 633 nm

provided by He/Ne lasers. Images of 1024 × 1024 pixels in size were acquired using a zoom factor of 5×.

Image analysis of CLSM micrographs was performed using ImageJ software. At least 10 representative images of each acid milk gel were chosen for image analysis processing. Two microstructural parameters of acid milk gels, porosity and tortuosity, were determined following the protocol developed previously (Silva, Legland, Cauty, Kolotuev, & Floury, 2015). The porosity is defined as the ratio between the aqueous phase area and the total area of the image and corresponds to the area fraction that are pixels represented in the image that do not contribute to the protein network of the gel. The tortuosity is defined as the ratio between the shortest path between two opposing borders of the image (i.e., avoiding the protein network) and the Euclidian distance between the same borders.

2.9. Water holding capacity of acid milk gels

Acid milk gels (20 g) were formed in situ in centrifuge tubes and centrifuged at 640 × *g* for 20 min at 4 °C as described previously (Karam, Gaiani, Hosri, Hussain, & Scher, 2015). The supernatant was collected and weighed. Measurements were taken in triplicate for each acid milk gel.

Water holding capacity (WHC) was calculated according to the following equation:

$$WHC\ (\%) = \left(1 - \frac{weight\ of\ supernatant}{weight\ of\ acid\ milk\ gel}\right) x\ 100$$

2.10. Textural properties of acid milk gels

Textural properties of acid milk gels were characterised using the back extrusion (pseudo-compression) method described previously (Ciron, Gee, Kelly, & Auty, 2010) with

some modifications. Back extrusion tests were performed using a Texture Analyser TA-XT2i (Stable Micro Systems Ltd., Godalming, Surrey, UK), equipped with a 5 kg load cell. Acid milk gels were produced in glass beakers (internal diameter = 50 mm; height = 65 mm) and stored at 4 °C. Measurements were carried out 18-24 h following gel formation and immediately after removal from storage at 4 °C. An extrusion disc (Ø = 35 mm), operating at a fixed test speed of 1.0 mm s^{-1}, to a depth of 25 mm, was used. The force-time curves were analysed using Texture Expert Exceed (Stable Micro Systems Ltd.). The textural parameters measured were: maximum positive force in compression (firmness); positive area of the curve, which indicates the internal strength of bonds within the product (consistency); maximum negative force of the curve, which indicates the force required to withdraw the probe from the sample (cohesiveness); and negative area of the curve (viscosity index). Triplicate measurements were taken on each acid milk gel.

2.11. Statistical analyses

One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were applied to the values obtained from the different analyses to determine which mean values were significantly different from one another at the 95% confidence level. Results are presented with the mean value ± standard deviation. The mean values were statistically compared using the R software (version R i386 3.0.2) (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Physical properties of milk protein suspensions

The apparent viscosity values of the unheated milk protein suspensions measured at 20 °C at a constant shear rate of 500 s^{-1} are presented in Table 2. This analysis was performed on the unheated suspensions at a relatively high shear rate to determine if the addition of MWP to the formulations influenced the viscosity of the suspensions during processing (e.g., pumping). The apparent viscosity of the unheated milk protein suspensions decreased significantly ($P < 0.05$) with increasing MWP content, even though there was an increase in the total solids content (caused mainly by the higher lactose content of the MWP ingredient) of the suspensions with increasing level of addition of MWP. The differences in apparent viscosity values can be explained by the average particle size in the suspensions, which increased with increasing level of addition of MWP (Table 2), leading to a lower extent of protein particle-particle interactions, and consequently to a decrease in the apparent viscosity (Krzeminski, Großhable, & Hinrichs, 2011); in addition, all of the particle size distribution profiles were mono-modal. Indeed, dispersions of MWP typically have protein aggregates in the diameter size range of 0.1–3.0 μm, with an average diameter of 1 μm (Bansal & Bhandari, 2016), much larger than the diameter of casein micelles in MPC (~202 nm; Silva & O'Mahony, 2016), which MWP is effectively replacing in formulations used in the current study.

Viscosity profiles for the milk protein suspensions during laboratory-scale HTST treatment are shown in Fig. 1. As expected, apparent viscosity values decreased with increasing temperature for all milk protein suspensions. The viscosity of milk and concentrated milk systems generally decreases with increasing temperature (Crowley, Dowling, Caldeo, Kelly, & O'Mahony, 2016; Horne, 1998; Joyce, Brodkorb, Kelly, & O'Mahony, 2016). Horne (1998) suggested that as the temperature of milk suspensions is increased, the strength of the hydrophobic interactions increases, the casein micelles are

tightened (i.e., become more compact), allowing the suspensions to flow more freely, contributing to a decrease in the apparent viscosity. The viscosity behaviour during heating (from 20 to 80 °C) was similar for all the suspensions, irrespective of the level of addition of MWP. However, during cooling (from 80 to 30 °C), and on holding at 30 °C at the end of the HTST process, the viscosity values were significantly ($P < 0.05$) higher for suspensions with higher levels of addition of MWP (Fig. 1).

3.2. Physical properties of acid milk gels

3.2.1. Small and large deformation rheological properties of acid milk gels

The effect of MWP addition on the changes in storage modulus (G′) with time during the formation of acid milk gels was studied using small amplitude oscillatory rheology (SAOR) and is shown in Fig. 2. The gelation time (GT) was generally longer for samples containing MWP compared with C, in agreement with Sturaro, Penasa, Cassandro, Varotto, and De Marchi (2014) who studied the effect of MWP addition on rennet coagulation of milk. In the current study, the longer gelation time for the samples containing MWP (GT for LS = 110 ± 1.7 min; MS = 108 ± 4.9 min; HS = 104 ± 5.4 min) compared with C (89 ± 1.6 min) can be explained mainly by the presence of MWP in those samples, which decreased the effective concentration of casein available for gel formation, and consequently the rate of aggregation of casein particles during gel formation, leading to delayed gelation. Moreover, the higher viscosity of the heated milk protein suspensions containing MWP (Fig. 1) would be expected to have slowed diffusion of protein during gelation and consequently slowed gel assembly compared with the control (C) sample. However, the TS sample presented a shorter gelation time (87 ± 0.3 min) and a higher gelation pH compared with the other samples. This observation may be explained by the absence of MPC in this sample, combined with a much

higher concentration of denatured/aggregated whey protein compared with the other samples. In addition, as the isoelectric points of the whey proteins (~5.2 to 4.8) are higher than those of the caseins (~4.6), the denatured whey proteins would be expected to aggregate at a higher pH than casein particles (Lucey, Teo, Munro, & Singh, 1997).

In general, G′ values during the formation of the acid gels were lower for the samples containing MWP (LS, MS, HS, TS) compared with C (Fig. 2). However, these changes were not directly related to the addition level of MWP, as the HS sample presented higher G′ values compared with the LS and MS samples. These results showed that low and medium level MWP addition were sufficient to significantly decrease ($P < 0.05$) the G′ values of the gels.

The TS sample showed a rapid increase in the G′ values during the early stages of acidification/gelation, followed by a reduction in the rate of increase at the later stages of the gelation process. Gels that have the ability to rearrange strongly at higher pH tend to be weaker (Chever et al., 2014), which can explain why the TS gel had lower G′ values at the end of acidification compared with the other samples (Fig. 2). Moreover, the formation of large denatured whey protein:casein aggregates in the samples containing high MWP content altered the gelation process during acidification of heated milk suspensions, leading to the formation of softer gels, in agreement with Lucey et al. (1997).

Large deformation rheological properties of the acid milk gels are shown in Fig. 3. At low shear strain values, the plots of shear stress as a function of shear strain were quite similar for the different acid milk gels. However, the shear stress at yielding was significantly ($P < 0.05$) lower for the acid milk gels containing MWP compared with the control sample. In addition, the shear stress and shear strain at yielding decreased significantly ($P < 0.05$) with increasing level of addition of MWP. These results confirmed that the TS gel was much softer compared with the other gels.

312

313 *3.2.2. Textural properties of acid milk gels*

314 Four textural parameters (firmness, consistency, cohesiveness and viscosity index)
315 were determined for the different acid milk gels and their values are shown in Table 3.
316 Textural parameters were measured in the gels that were stored at 4 °C and were in good
317 agreement with the large deformation rheological properties of the gels measured at 30 °C.
318 Indeed, these results clearly showed that the addition of MWP led to the formation of softer
319 acid milk gels compared with the control sample. These results are consistent with findings of
320 other authors reporting softening of the texture of yoghurts or cheeses with the addition of
321 MWP (Lobato-Calleros et al., 2007; Sahan et al., 2008; Sandoval-Castilla et al., 2004;
322 Schenkel et al., 2013; Torres et al., 2016). In addition, the values for the textural parameters
323 generally decreased with increasing MWP content, although these differences were not
324 always significant ($P < 0.05$) between LS, MS and HS. The TS gel presented much lower
325 values for textural parameters compared with the other gels. The reasons for the softness of
326 the TS gel compared with the others are related to the higher gelation pH and the rheological
327 behaviour of this sample during the acidification process, as previously discussed.

328

329 *3.2.3. Microstructural properties and water holding capacity of acid milk gels*

330 The CLSM technique was used to visualise the protein microstructural network in the
331 acid milk gels obtained from acidification of heated milk protein suspensions containing
332 different MWP contents (from 0% to 4.4%, w/w) (see Fig. 4). The CLSM micrographs
333 showed that the control gel presented a relatively fine network, containing numerous small
334 pores. The addition of MWP contributed to a more open structure due to the creation of new
335 serum channels in the acid milk gels. The inclusion of the MWP particles increased the
336 openness of the gel, inhibiting the aggregation of casein particles, in agreement with previous

studies (Sturaro et al., 2014; Torres et al., 2011). This can help explain the softness of the acid milk gels containing MWP, which were less elastic and less firm compared with the control gel. In an analogous manner, McMahon et al. (1996) showed that when large serum channels are distributed throughout the protein network of Mozzarella cheese, the cheese is softer and more pliable because of the increased moisture and decreased coalescence of the protein strands. In the TS gel, the presence of large whey protein aggregates, due to the high level of addition of MWP, seemed to interfere strongly with the homogeneity of the network and regularity of the protein phase, leading to excessive softening (Krzeminski et al., 2011; Schenkel et al., 2013).

To confirm these differences in coarseness perceived qualitatively for the different acid milk gels, image analysis was applied to a series of obtained micrographs for each sample and two microstructural parameters (porosity and tortuosity) were measured. Porosity and tortuosity values for the different acid milk gels are shown in Table 3. The porosity of the acid milk gels increased significantly ($P < 0.05$) and the tortuosity decreased significantly ($P < 0.05$) with increasing level of addition of MWP. The quantification of these microstructural parameters confirmed that the addition of MWP allowed the formation of cavities (i.e., new serum channels) in the acid milk gel protein network structures, leading to a more porous and open structure, characterised by a greater connectivity between the pores, i.e., lower tortuosity.

The WHC of the acid milk gels decreased significantly ($P < 0.05$) with increasing MWP content (Table 3), in agreement with Purwanti et al. (2013). Purwanti et al. (2013) used whey protein isolate (WPI) as a model protein in high protein, concentrated suspensions and observed that the micro-structuring introduced domains with different protein contents, and hence, provided a driving force for migration of water and other components. Something similar was observed in the present study for the acid milk gels containing MWP compared

with the control gel. This can be explained by the more open structure and higher porosity of the samples containing higher levels of addition of MWP, which facilitated greater expulsion of the serum phase during centrifugation compared with the control sample gel. These results showed that the WHC of the gels can be explained by this length scale evaluation of gel microstructure. Another factor that may explain the lower WHC of the acid milk gels with higher levels of addition of MWP is the increased ratio of denatured to native whey protein. Torres et al. (2011) showed that yoghurt systems with a greater proportion of denatured whey proteins provided gels with lower WHC. Moreover, with increasing MWP content, the samples contain a lower ratio of casein to non-casein protein, which could also contribute to the lower WHC of the gels containing MWP compared with the control gel. Moreover, while other authors (Meletharayil, Patel, Metzger, & Huppertz, 2016) have observed that the properties of acid gels are influenced by the lactose content of reconstituted protein systems, it is not believed that the slight increase in lactose content with increasing levels of incorporation of MWP could explain the differences in WHC measured in the present study.

4. Conclusions

This study showed that the incorporation of MWP in fat-free, high-protein milk systems influenced the rheological, microstructural, textural and water holding properties of the resulting acid gels. The acid milk gels containing MWP had lower storage modulus, lower yield stress, lower firmness, higher porosity, lower tortuosity and lower water holding capacity compared with the control gel (made without MWP). The incorporation of MWP is a promising approach to modulate the microstructure of high-protein acid milk gels. These

results are useful in the development of low-fat, high-protein fresh dairy products with tailored and desired textural and functional characteristics.

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Figure legends

Fig. 1. Viscosity during laboratory-scale HTST treatment of the milk protein suspensions: ○, control; ▲, low MWP; ◆, medium MWP; ■, high MWP; ●, total MWP. The temperature profile is shown by the broken line. Values are means from replicates ($n \geq 3$).

Fig. 2. Storage modulus (G′) as a function of incubation time post addition of GdL during the formation of acid milk gels made by acidification of the heated milk protein suspensions: ○, control; ▲, low MWP; ◆, medium MWP; ■, high MWP; ●, total MWP. Values are means from replicates ($n \geq 3$).

Fig. 3. Shear stress as a function of applied deformation at a constant shear rate (0.01 s^{-1}) for acid milk gels made by acidification of the heated milk protein suspensions with GdL at 30 °C: ○, control; ▲, low MWP; ◆, medium MWP; ■, high MWP; ●, total MWP. Values are means from replicates ($n \geq 3$).

Fig. 4. Representative CLSM micrographs of acid milk gels made by acidification of the heated milk protein suspensions with GdL at 30 °C: (a) control, (b) low MWP, (c) medium MWP, (d) high MWP and (e) total MWP.

Table 1

Protein composition of reconstituted milk protein suspensions obtained from reconstitution of skim milk powder, milk protein concentrate and microparticulated whey protein in deionised water. [a]

Suspension	Protein composition (%, w/w)		
	SMP	MPC	MWP
C	5.70	2.30	0.00
LS	5.70	2.01	0.29
MS	5.70	1.72	0.58
HS	5.70	1.15	1.15
TS	5.70	0.00	2.30

[a] Abbreviations are: SMP, skim milk powder; MPC, milk protein concentrate; MWP, microparticulated whey protein; C, control; LS, low MWP; MS, medium MWP; HS, high MWP; TS, total MWP.

Table 2

Apparent viscosity and protein particle size of unheated milk protein suspensions. [a]

Unheated suspension	Apparent viscosity (mPa s)	Protein particle size (nm)
C	$7.46^{d} \pm 0.02$	$211^{a} \pm 2$
LS	$6.97^{c} \pm 0.02$	$212^{a} \pm 2$
MS	$6.96^{c} \pm 0.04$	$214^{b} \pm 4$
HS	$6.51^{b} \pm 0.04$	$219^{c} \pm 4$
TS	$6.34^{a} \pm 0.01$	$228^{d} \pm 4$

[a] Abbreviations are: C, control; LS, low microparticulated whey protein (MWP); MS, medium MWP: HS, high MWP; TS, total MWP. Values are means ($n \geq 3$) ± standard deviation; means with different superscript letters in the same column are significantly different ($P < 0.05$).

Table 3

Textural parameters (firmness, consistency, cohesiveness and viscosity index), microstructural parameters (porosity and tortuosity) and water holding capacity of acid milk gels. [a]

Acid milk gel	Firmness (N)	Consistency (N s)	Cohesiveness (N)	Viscosity index (N s)	Porosity	Tortuosity	WHC (%)
C	$3.6^{c} \pm 0.2$	$89.9^{c} \pm 4.0$	$1.7^{c} \pm 0.0$	$14.7^{b} \pm 0.5$	$0.48^{a} \pm 0.03$	$1.50^{e} \pm 0.27$	$93.1^{e} \pm 0.2$
LS	$3.3^{b} \pm 0.0$	$83.6^{b} \pm 2.0$	$1.6^{b} \pm 0.0$	$12.8^{ab} \pm 0.1$	$0.52^{b} \pm 0.05$	$1.25^{d} \pm 0.13$	$92.5^{d} \pm 0.1$
MS	$3.2^{b} \pm 0.1$	$83.0^{b} \pm 2.4$	$1.6^{b} \pm 0.1$	$14.5^{b} \pm 0.3$	$0.56^{c} \pm 0.01$	$1.19^{c} \pm 0.04$	$92.2^{c} \pm 0.1$
HS	$3.2^{b} \pm 0.1$	$80.9^{b} \pm 2.4$	$1.6^{b} \pm 0.1$	$13.5^{ab} \pm 0.4$	$0.65^{d} \pm 0.03$	$1.10^{b} \pm 0.03$	$91.8^{b} \pm 0.1$
TS	$2.5^{a} \pm 0.0$	$68.1^{a} \pm 2.2$	$1.4^{a} \pm 0.0$	$12.0^{a} \pm 0.7$	$0.71^{e} \pm 0.02$	$1.06^{a} \pm 0.02$	$91.1^{a} \pm 0.3$

[a] Abbreviations are: WHC, water holding capacity; C, control (C), LS, low microparticulated whey protein (MWP); MS, medium MWP: HS, high MWP; TS, total MWP. The values are means ($n \geq 3$) ± standard deviation; means with different superscript letters in the same column are significantly different ($P < 0.05$).

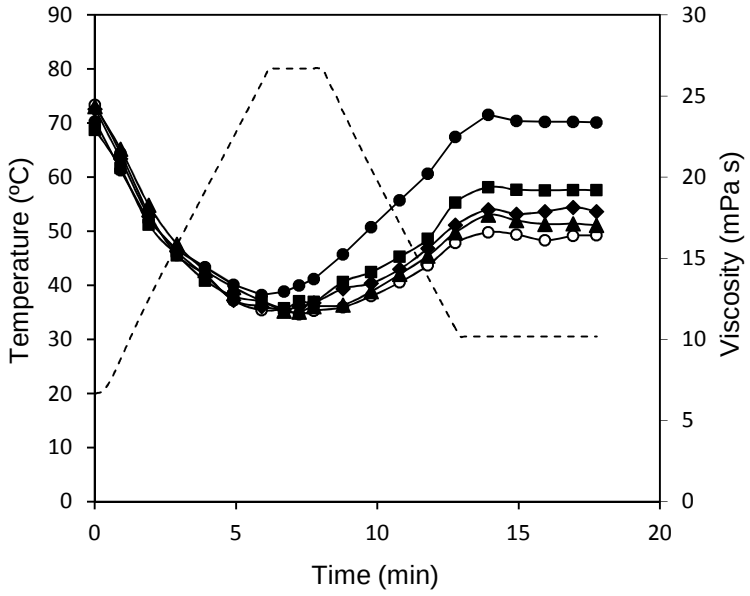

Figure 1

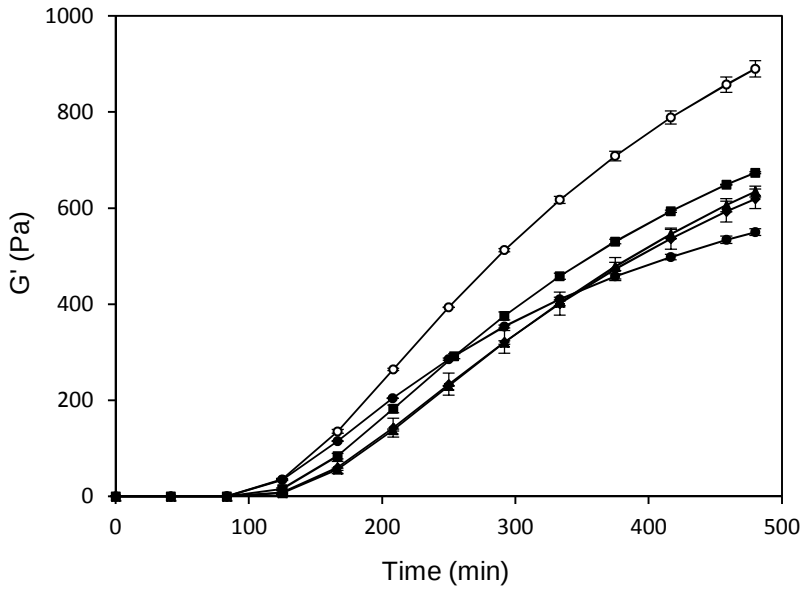

Figure 2

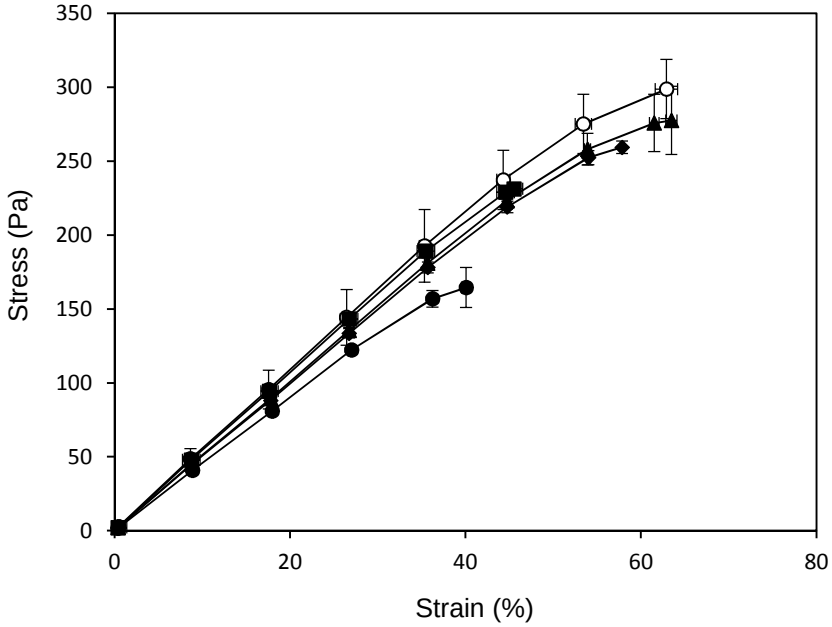

Figure 3

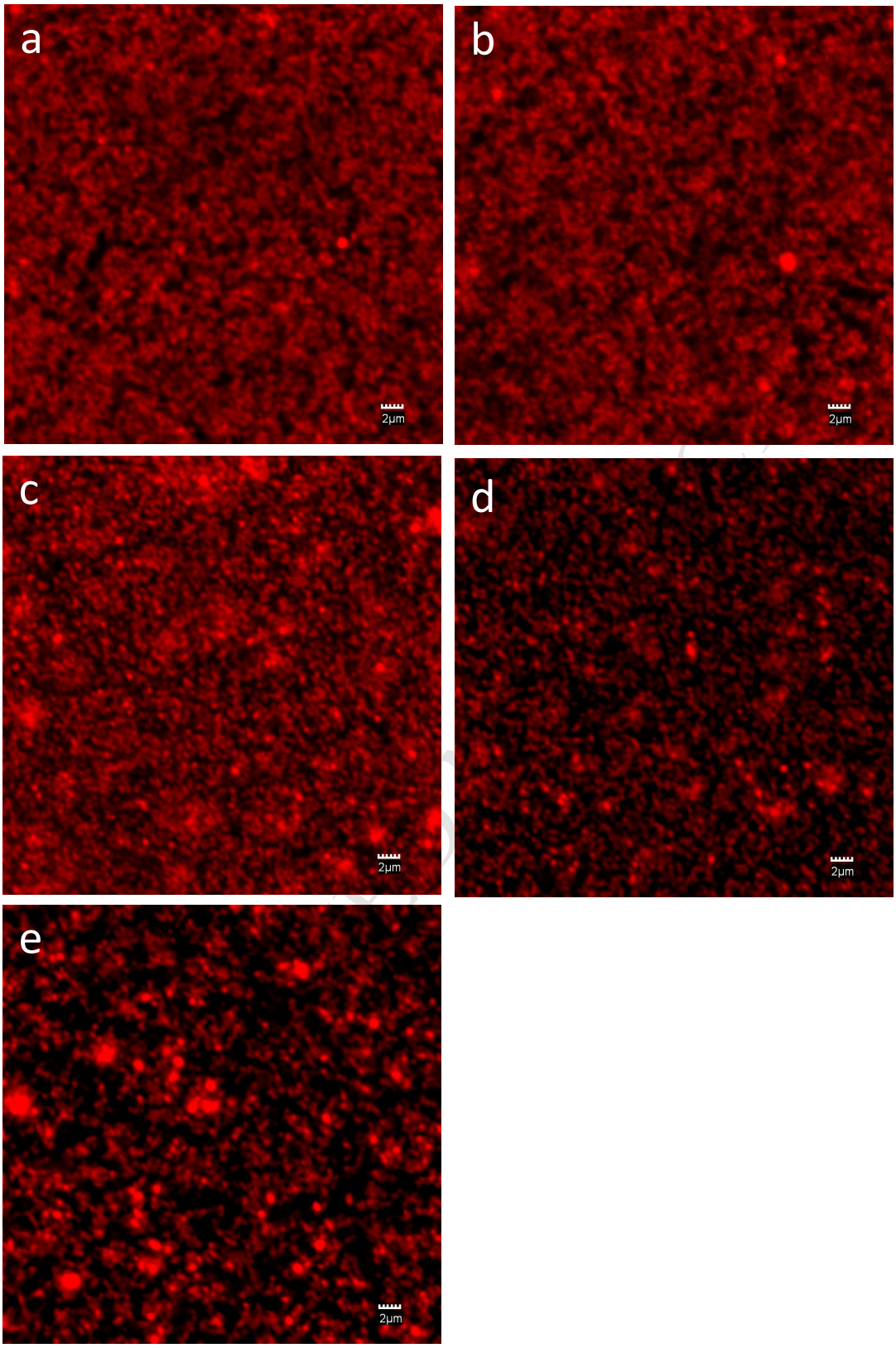

Figure 4